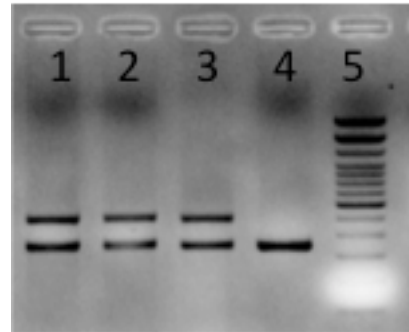


MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	37375
MMRRC Strain Name	B6J.B6N- <i>Ube3a</i> ^{tm1a(KOMP)Wtsi} /Mmnc
Gene Name(s)	ubiquitin protein ligase E3A
Breeding Protocol(s)	Backcross
Protocol Date	8/16/2022

PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	0.5
DNA	2



1. Heterozygous Sample
2. Heterozygous Sample
3. Heterozygous Sample
4. Wild-type Control
5. 100bp Ladder (Invitrogen)

Thermal Cycler:

Step 1: 94C, 5min
 Step 2: 94C, 45sec
 Step 3: 55C, 45sec
 Step 4: 72C, 60sec
 Step 2 to 4 Cycles: 30
 Step 5: 72C, 7min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 352bp MUTANT: 520bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

Ube(37375)F: AAAATTGGGTATGCGAGCTG

Ube(37375)R: GGGTCTAAGGGCCTATGAA